therapy, rifaximin did not select for significant resistance among gut flora when given for 3 days. It is unlikely that rifaximin would stimulate the development of rifampicin-resistant *Mycobacterium tuberculosis*, since the drug remains largely in the gut during short-term use for the treatment of diarrhoea. Extra-intestinal tissues infected with *M. tuberculosis* should not be exposed to significant concentrations of the drug, and growth of *M. tuberculosis* on media containing varying concentrations of rifaximin does not lead to the selection of rifampicin-resistant strains [11].

In summary, rifaximin appears to be a suitable drug for the management of travellers' diarrhoea [12] and other non-systemic, non-dysenteric enteric bacterial infections. This drug has now been licensed for use in the USA.

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REFERENCES

1. Steffen R, Sack DA, Riopel L *et al.* Therapy of travelers' diarrhea with rifaximin on various continents. *Am J Gastroenterol* 2003; **98**: 1073–1078.
2. Murray BE, Rensimer ER, DuPont HL. Emergence of high-level trimethoprim resistance in fecal *Escherichia coli* during oral administration of trimethoprim/sulfamethoxazole. *N Engl J Med* 1982; **306**: 130–135.
3. Gomi H, Jiang Z-D, Adachi JA *et al.* In vitro antimicrobial susceptibility testing of bacterial enteropathogens causing traveler's diarrhea in four geographic regions. *Antimicrob Agents Chemother* 2001; **45**: 212–216.
4. Thornsberry C, Hill BC, Swenson JM, McDougal LK. Rifampin: spectrum of antibacterial activity. *Rev Infect Dis* 1983; **5**(suppl 3): S412–S417.
5. Farr BM. Rifamycins. In: Mandell GL, Bennett JE, Dolin R, eds. *Principles and practice of infectious diseases*, 5th edn. Philadelphia: Churchill Livingstone, 2000; 348–361.
6. Hoover W, Gerlach E, Hoban D, Eliopoulos G, Pfaller M, Jones R. Antimicrobial activity and spectrum of rifaximin, a new topical rifamycin derivative. *Diagn Microbiol Infect Dis* 1993; **16**: 111–118.
7. Gillis J, Brogden R. Rifaximin: a review of its antibacterial activity, pharmacokinetic properties and therapeutic potential in conditions mediated by gastrointestinal bacteria. *Drugs* 1995; **49**: 467–484.
8. Corazza GR, Ventrucci M, Strocchi A *et al.* Treatment of small intestine bacterial overgrowth with rifaximin, a non-absorbable rifamycin. *J Int Med Res* 1988; **16**: 312–316.
9. Miglio F, Valpiani D, Ricca Rossellini S, Ferrieri A. Rifaximin, a nonabsorbable rifamycin, for the treatment of hepatic encephalopathy: a double-blind randomized trial. *Curr Med Res Opin* 1997; **13**: 593–601.
10. Beseghi U, De'Angelis GL. Comparison of two non-absorbable antibiotics for treatment of bacterial enteritis in children. *Eur Rev Med Pharmacol Sci* 1997; **2**: 131–136.
11. Soro O, Pesce A, Raggi M, Debbia EA, Schito GC. Selection of rifampicin-resistant *Mycobacterium tuberculosis* does not occur in the presence of low concentrations of rifaximin. *Clin Microbiol Infect* 1997; **3**: 147–151.
12. DuPont HL, Ericsson CD. Prevention and treatment of traveler's diarrhea. *N Engl J Med* 1993; **328**: 1821–1827.

RESEARCH NOTE

Production by *Escherichia coli* isolates of siderophore and other virulence factors and their pathogenic role in a cutaneous infection model

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ABSTRACT

Escherichia coli isolates from urinary tract infections (UTIs) (n = 124), extra-urinary sites (n = 37) and normal faecal samples (n = 51) were examined for the presence of virulence factors, including siderophores (aerobactin and enterobactin). The proportion of aerobactin producers was significantly higher in UTI (69.4%; p 0.001) and extra-urinary samples (70.3%; p 0.007) than in controls (41.2%), while the proportion of enterobactin producers was significantly lower in the UTI samples than in the controls (p 0.027). In a cutaneous infection model, aerobactin-positive *E. coli* showed more growth than non-aerobactin and non-enterobactin isolates, even when other virulence factors were identical.

Keywords Aerobactin, cutaneous infection model, enterobactin, *Escherichia coli*, siderophore, virulence factors

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Escherichia coli is one of the commonest microorganisms found in urinary tract infections (UTIs). Bacterial virulence is multifactorial, and the virulence characteristics of *E. coli* have been studied in UTIs and bacteraemia [1–3]. Among the proposed virulence factors are fimbrial adhesins, haemolysin, siderophores, serum resistance and inactive serum resistance [4–8]. Iron compounds are known to increase the growth of bacteria, and enteric bacteria produce a catechol-type siderophore, enterobactin, and a hydroxamate-type siderophore, aerobactin, under conditions of iron starvation *in vitro* [9–11]. The aim of this study was to investigate the production of siderophores and other virulence factors by *E. coli* isolates, and to investigate the role of siderophores in pathogenicity with a cutaneous infection model.

E. coli isolates from UTIs (n = 124) and from extra-urinary infection sites (n = 37) were studied. UTI was defined as $>10^5$ CFU/mL of urine. Control isolates (n = 51) were obtained from the faeces of healthy adults. All isolates were collected at the Pamukkale University Hospital of Denizli, Turkey.

The method of Rabsch and Reissbrodt [10,11] was used for detection of aerobactin and enterobactin, with Tris-succinate medium for aerobactin and Vogel–Bonner medium for enterobactin. Haemagglutination and mannose-resistant haemagglutination of human group O erythrocytes was tested with a microplate haemagglutination technique. Detection of haemolysin production, a serum resistance assay and tests for survival of *E. coli* in inactivated serum were performed as described previously [6–8].

To evaluate pathogenicity in mice, isolates were selected that varied in siderophore status, but which were all haemolysin-negative, mannose-resistant haemagglutination-positive, and serum- and inactive serum-resistant. BALB-c mice weighing 25–30 g were assigned randomly to the following groups: (1) enterobactin-positive (E^+) (n = 5); (2) aerobactin-positive (A^+) (n = 5); (3) aerobactin- and enterobactin-positive (AE^+) (n = 5); and (4) aerobactin- and enterobactin-negative (AE^-) (n = 5). Bacterial suspensions (200 μL of 10^8 CFU/mL) were injected subcutaneously. After 24 h, abscesses and inflamed tissues were removed and weighed. The tissues were homogenised under sterile conditions, and then diluted with 5 mL of sterile phosphate-buffered saline. The diluted homogenates were inoculated (10 μL) on to blood and eosin methylene blue agar and incubated at 37°C. After 24 h, the number of bacteria/g of tissue was estimated.

For statistical analysis, the chi-square test with continuity correction was used. The Kruskal–Wallis and Mann–Whitney *U*-tests were used for the subcutaneous infection model. A p value of <0.05 was considered significant.

The proportion of aerobactin producers among the control faecal isolates (41.2%) was significantly lower than for the UTI isolates (69.4%; p 0.001) and extra-urinary samples (70.3%; p 0.007) (Table 1). In contrast, the proportions of enterobactin producers were 44.4%, 51.4% and 62.7% for UTI, extra-urinary and faecal isolates, respectively; this was significantly lower for the UTI isolates than for the controls (p 0.027). The presence of other virulence factors is shown in Table 1.

In the cutaneous infection model, *E. coli* growth in the A^+ group was significantly higher than in the AE^- group, and growth in the AE^+ group was significantly higher than in the AE^- group (p 0.028 and p 0.021, respectively) (Table 2). The number of E^+ isolates was higher than that of AE^- isolates, and the number of A^+ isolates was higher than that of E^+ isolates, but neither difference reached statistical significance (Table 2).

Table 1. Distribution of virulence factors among *Escherichia coli* isolates from urinary tract infections, extra-urinary sites and control faecal samples

Virulence factors	% UTI isolates (n = 124)	% Extra-urinary isolates (n = 37)	% Control isolates (n = 51)
Aerobactin	69.4 (p 0.001)	70.3 (p 0.007)	41.2
Enterobactin	44.4 (p 0.027)	51.4	62.7
Haemolysin	33.9 (p 0.001)	35.1 (p 0.004)	9.8
HA-positive	83.9 (p 0.011)	78.4	66.7
MRHA	56.5 (p 0.001)	54.1 (p 0.020)	29.4
Serum resistance	62.1	59.5	66.7
Inactivated serum resistance	87.1	75.7 (p 0.031)	92.2

UTI, urinary tract infection; HA, haemagglutination; MRHA, mannose-resistant haemagglutination.

Table 2. Growth of *Escherichia coli* in the subcutaneous infection model according to virulence factor status

		Growth ($\log_{10}$ CFU/g)		
Virulence factor status	n	Median	SEM	Range
Aerobactin- and enterobactin-negative (AE^-)	5	4.52	1.25	0–5.48
Aerobactin-positive (A^+)	5	6.90[a,b]	0.39	5.15–7.10
Enterobactin-positive (E^+)	5	5.39[c]	1.17	0–6.80
Aerobactin- and enterobactin-positive (AE^+)	5	5.69[d]	0.31	5.33–7.10

[a]p 0.028 vs. AE^-; [b]p 0.07 vs. E^+ [c]p 0.245 vs. AE^-; [d]p 0.021 vs. AE^-.

The presence of siderophores and other virulence factors in Gram-negative bacteria has been investigated previously [11–14]. In the present study, the proportion of aerobactin-producing *E. coli* isolates was significantly higher in the UTI and extra-urinary samples, which is consistent with the results of other studies [14,15]. Ruiz *et al.* [2] found that production of aerobactin in *E. coli* isolates from patients with nephritis was higher than in isolates from patients with cystitis. It was suggested that siderophore production might be an important factor in the progression of prostatitis and pyelonephritis. Vila *et al.* [16] found that the *iucD* gene was more common in *E. coli* isolates causing pyelonephritis than in isolates causing cystitis.

In pathogenicity studies, intraperitoneal, subcutaneous and intradermal routes of inoculation have been used, as well as the ascending UTI model [3,17–19]. The role of siderophores was investigated in the present study with the subcutaneous route, as it was quick, easy and practical. The role of aerobactin and other virulence factors in pathogenicity has been studied previously in mice [8,18,19]. Torres *et al.* [19] suggested that TonB was required for iron uptake by uropathogenic *E. coli* in a mouse model of UTI, as TonB-negative mutant isolates showed reduced virulence. In a competitive assay, aerobactin and enterobactin transport-defective isolates did not grow in the kidney, and aerobactin transport-defective but enterobactin-intact isolates did not grow together with the wild-type isolates in the kidney.

In the mouse model used in the present study, *E. coli* A^+ isolates showed more growth than the AE^- isolates, and AE^+ isolates showed more growth than the AE^- isolates. *E. coli* A^+ isolates showed more growth than E^+ isolates, but the difference was not statistically significant. Montgomerie *et al.* [8] suggested that aerobactin is a more significant factor in bacteraemia in mice than enterobactin. In another study, *E. coli* isolates were assigned to three groups according to LD_{50}. In the most virulent group, aerobactin production was 88%, compared to 24% in avirulent isolates [20]. In the ascending pyelonephritis mouse model, siderophore-producing isolates have increased renal pathogenicity [21]. It has also been shown that the number of aerobactin-producing *E. coli* isolates decreases in the presence of mouse monoclonal antibody to ferric aerobactin in newborn calf sera [22].

In the present study, isolates that differed by siderophore status were compared. Although many virulence factors, including siderophores, mannose-resistant fimbriae, haemolysin and serum resistance, have important roles in pathogenesis, it was observed that specific siderophore production affected extra-intestinal *E. coli* infection, even when other virulence factors were identical. The present study indicated that siderophores have an important role in the progress of infection, and that production of aerobactin may be more important than production of enterobactin. Further studies with animal models are necessary to clarify this.

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REFERENCES

1. Johnson JR, Stell AL. Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. *J Infect Dis* 2000; **181**: 261–272.
2. Ruiz J, Simon K, Horcajada JP *et al.* Difference in virulence factors among clinical isolates of *Escherichia coli* causing cystitis and pyelonephritis in women and prostatitis in men. *J Clin Microbiol* 2002; **40**: 4445–4449.
3. Blanco J, Alonso MP, Gonzales EA, Blanco M, Garabal JI. Virulence factors of bacteraemic *Escherichia coli* with particular reference to production of cytotoxic necrotising factor (CNF) by P-fimbriate strains. *J Med Microbiol* 1990; **31**: 175–183.
4. Svanborg C, Godaly G. Bacterial virulence in urinary tract infection. *Infect Dis Clin North Am* 1997; **11**: 513–529.
5. Johnson JR. Virulence factors of *Escherichia coli* urinary tract infection. *Clin Microbiol Rev* 1991; **4**: 80–128.
6. Valvano MA, Silver RP, Crosa JH. Occurrence of chromosome- or plasmid-mediated aerobactin iron transport systems and hemolysin production among clonal groups of human invasive strains of *Escherichia coli* K1. *Infect Immun* 1986; **52**: 192–199.
7. Puzova H, Siegfried L, Kmetova M, Filka J, Takacova V, Durovicova J. Fimbriation, surface hydrophobicity and serum resistance in uropathogenic strains of *Escherichia coli*. *FEMS Immunol Med Microbiol* 1994; **9**: 223–230.
8. Montgomerie JZ, Bindereif A, Neilands JB, Kalmanson GM, Guze LB. Association of hydroxamate siderophore (aerobactin) with *Escherichia coli* isolated from patients with bacteremia. *Infect Immun* 1984; **46**: 835–838.

9. Neilands JB. Siderophores: structure and function of microbial iron transport compounds. *J Biol Chem* 1995; **270**: 26723–26726.
10. Rabsch W, Reissbrodt R. Investigations of *Salmonella* strains from different clinical-epidemiological origin with phenolate and hydroxamate (aerobactin) siderophore bioassays. *J Hyg Epidemiol Microbiol Immunol* 1988; **32**: 353–360.
11. Rabsch W, Reissbrodt R. Biotest zum Nachweis von hydroxamat-Fe-chelatoren (Aerobactin). *J Basic Microbiol* 1985; **25**: 663–667.
12. Ang-Kucuker M, Kucukbasmaci O, Tekin M, Akbulut D, Buyukbaba-Boral O, Ang O. Serotypes, siderophore synthesis, and serum resistance of uropathogenic *Klebsiella* isolates. *Adv Exp Med Biol* 2000; **485**: 237–241.
13. Marrs CF, Zhang L, Tallman P *et al*. Variations in 10 putative uropathogen virulence genes among urinary, faecal and peri-urethral *Escherichia coli*. *J Med Microbiol* 2002; **51**: 138–142.
14. Carbonetti NH, Boonchal S, Parry SH, Vaisanen-Rhen V, Korhonen TK, Williams PH. Aerobactin-mediated iron uptake by *Escherichia coli* isolates from human extraintestinal infections. *Infect Immun* 1986; **51**: 966–968.
15. Vidotto MC, Furlaneto MC, Perugini MRE. Virulence factors of *Escherichia coli* in urinary isolates. *Brazil J Med Biol Res* 1991; **24**: 365–373.
16. Vila J, Simon K, Ruiz J *et al*. Are quinolone-resistant uropathogenic *Escherichia coli* less virulent? *J Infect Dis* 2002; **186**: 1039–1042.
17. Brook I. Enhancement of growth of aerobic and facultative bacteria in mixed infections with *Bacteroides* species. *Infect Immun* 1985; **50**: 929–931.
18. Morin MD, Hopkins WJ. Identification of virulence genes in uropathogenic *Escherichia coli* by multiplex polymerase chain reaction and their association with infectivity in mice. *Urology* 2002; **60**: 537–541.
19. Torres AG, Redford P, Welch RA, Payne SM. Ton-B dependent systems of uropathogenic *Escherichia coli*: aerobactin and heme transport and Ton B are required for virulence in the mouse. *Infect Immun* 2001; **69**: 6179–6185.
20. Ou Said AM, Contrepois MG, Der Vartanian M, Girardeau JP. Virulence factors and markers in *Escherichia coli* from calves with bacteremia. *Am J Vet Res* 1988; **49**: 657–660.
21. Sharma S, Harjai K, Mittal R. Enhanced siderophore production and mouse kidney pathogenicity in *Escherichia coli* grown in urine. *J Med Microbiol* 1991; **35**: 325–329.
22. Roy D, Expert D, Razafındratsita A *et al*. Activity and specificity of a mouse monoclonal antibody to ferric aerobactin. *Infect Immun* 1992; **60**: 768–772.

RESEARCH NOTE

Mycobacterial testing in hospital laboratories: results from a questionnaire survey in Italy

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ABSTRACT

Between 1999 and 2001, 355 hospital laboratories in Italy were asked to complete a questionnaire addressing mycobacterial test methods, 1-year workloads and laboratory safety features. Analysis of the data showed that rapid methods for mycobacterial testing were being used by most larger laboratories; however, sub-optimal methods were still in use in small and medium-size laboratories. In a country such as Italy, which has a low prevalence of tuberculosis cases, implementation of rapid technologies, combined with regionalisation of mycobacterial diagnostic services, seems to be the most reasonable and cost-effective strategy.

Keywords Diagnostic tests, *Mycobacterium tuberculosis*, rapid methods, safety, survey

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Tuberculosis (TB) may be considered a global emergency, with >2 million people dying and 8 million new cases each year [1]. Although the prevalence of TB in the industrialised world is relatively low, outbreaks caused by multiresistant strains of the *Mycobacterium tuberculosis* complex (MTB) have occurred in hospitals, prisons and shelters for homeless people, often involving

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